

## Claims:

1. A method of determining the presence of a nucleic acid in a sample comprising the steps of
  - 5 providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid, amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity, measuring fluorescence intensity of the fluorescent entity at each of the
  - 10 plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle, obtaining an individual score from each of a plurality of tests, the plurality of tests comprising a Confidence Interval Test and a Signal-to-Noise Ratio Test, and using the scores to ascertain whether the nucleic acid is present in the
  - 15 sample.
2. The method of claim 1 wherein the plurality of tests further comprise a Channel Consistency Test and an Efficiency Test.
- 20 3. The method of claim 2 wherein the plurality of tests further comprise a Function Ordering Test, a Maximum to Baseline Comparison Test, and a Last Rise Test.
4. The method of claim 3 wherein the individual scores are each
  - 25 corrected with a predetermined correction factor, and wherein the using step comprises generating a *Score*, wherein *Score* comprises the product of each of the corrected individual scores, divided by a predetermined threshold value.
5. The method of claim 4 wherein the *Score* is generated according to
  - 30 the formula

$$Score = \frac{(T_1^{P_1})(T_2^{P_2}) \dots (T_n^{P_n})}{Threshold}$$

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6. The method of claim 3 wherein the using step comprises generating a *CallValue*, wherein the *CallValue* comprises the sum of the logarithm of each of the individual scores.

7. The method of claim 6 wherein the *CallValue* is generated according to the formula

$$CallValue = \sum P_i \log T_i - \log(Threshold)$$

wherein  $P_i$  is a correction factor chosen for each of the Tests,  $T_i$  is the score from each of the tests, and *Threshold* has a value chosen to provide a convenient dividing point between positive and negative calls.

8. The method of claim 7 wherein the sample is called positive if *CallValue* > 1 and the sample is called negative if *CallValue* < -1.

9. The method of claim 3, further comprising the steps of determining whether the sample has a Late-Rise positive signal, and performing additional amplification cycles.

10. The method of claim 1 wherein the plurality of tests further comprise at least one test selected from the group consisting of a Channel Consistency Test, an Efficiency Test, a Function Ordering Test, a Maximum to Baseline Comparison Test, and a Last Rise Test.

11. The method of claim 1 wherein the presence of a nucleic acid is further verified by melting temperature analysis.

12. The method of claim 1 wherein the using step comprises

generating a *CallValue*, wherein the *CallValue* comprises the sum of the logarithm of each of the individual score

13. The method of claim 12 wherein the *CallValue* is generated  
5 according to the formula

$$CallValue = P_1 \log T_1 + P_2 \log T_2 - \log(Threshold)$$

wherein:

- 10  $T_1$  is the score from the Signal-to-Noise Ratio Test,  
 $T_2$  is the score from the Confidence Interval Test,  
 $P_1$  is a correction factor for the Signal-to-Noise Ratio Test,  
 $P_2$  is a correction factor for the Confidence Interval Test, and  
 $Threshold$  has a value chosen to provide a convenient dividing point  
between positive and negative calls.

- 15 14. The method of claim 13 wherein the value of *Threshold* is chosen  
to maximize the greatest number of correct positive calls when *CallValue* > 0 and to  
maximize the greatest number of correct negative calls when *CallValue* < 0.

- 20 15. The method of claim 13 wherein  
 $T_1$  is calculated according to the formula

and

- 25  $T_2$  is calculated according to the formula

$$T_2 = \frac{\sum (F_j - L(j))^2}{NoiseLevel}$$

16. The method of claim 15 wherein  
 $P_1$  is between -6.0 and -4.0,

$P_2$  is between 0.5 and 1.0, and

*Threshold* is between 1.5 and 2.0.

17. A method of determining the presence of a nucleic acid in a sample comprising the steps of
- providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid, amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,
  - measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,
  - obtaining a score from each of a plurality of tests, each of the plurality of tests using the fluorescence values to generate the score, and
  - using the scores to ascertain whether the nucleic acid is present in the sample.

18. A device for determining the presence of a nucleic acid in a sample comprising
- an instrument for temperature cycling to amplify the nucleic acid,
  - a fluorimeter for detecting fluorescence during amplification of the nucleic acid, the fluorescence obtained from a fluorescent entity capable of providing a signal related to the quantity of the nucleic acid, and
  - a processor for performing analysis routines, wherein the processor is programmed to obtain a score from each of a plurality of tests, each of the plurality of tests using fluorescence values measured by the fluorimeter to generate the score, and to use the scores to ascertain whether the nucleic acid is present in the sample.

19. The device of claim 18 wherein the plurality of tests comprise a Confidence Interval Test and a Signal-to-Noise Ratio Test.

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20. The device of claim 19 wherein the plurality of tests further comprise a Channel Consistency Test and an Efficiency Test.

21. The device of claim 20 wherein the plurality of tests further  
5 comprise a Function Ordering Test, a Maximum to Baseline Comparison Test, and a Last Rise Test.

22. The device of claim 18 wherein the instrument is configured for  
10 rapid thermal cycling.

23. The device of claim 22 wherein the instrument employs capillary  
tubes and hot air control.

24. The device of claim 18 provided in a portable container for field  
15 use.

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